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DETECTION OF *AEROMONAS HYDROPHILA* IN RAW MILK AND SOME MILK PRODUCTS WITH REFERENCE TO ITS PUBLIC HEALTH HAZARD

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ABSTRACT

This study aimed to determine *Aeromonas spp*. in raw milk and some milk products. A total of 100 raw milk, kareish cheese, ice cream and baladi yoghurt (25 samples, each) were collected from different dairy shops and street peddlers in Assiut city, Egypt and were bacteriologically examined for presence and count of *Aeromonas spp*. The incidences of counted *Aeromonas spp*. in raw milk, kareish cheese, ice cream and baladi yoghurt were 36, 32, 24 and 0.0%, respectively, with average counts of 1.0×10^5, 3.2×10^4, 5.0×10^2 and < 100 cfu/ml, respectively. The incidences of counted *Aeromonas hydrophila*, in raw milk, kareish cheese and ice cream were 16, 12 and 8%, while for *Aeromonas caviae*, the incidences were 12, 16 and 12%, respectively. Moreover, the incidences of counted *Aeromonas sobria* in raw milk, kareish cheese and ice cream were 8, 4 and 4%, respectively. Baladi yoghurt samples were negative for *Aeromonas spp.* in this study. All the recovered *Aeromonas hydrophila* organisms were confirmed by PCR assay for the presence of *16S* rRNA gene and 100% of the tested strains harboured this gene. The *aer*A and *ahh*1 virulence genes were present in *Aeromonas hydrophila* in percentages of 66.67 and 77.78%, respectively. All the recovered *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* strains, in this study, exhibited 100% virulence properties on bases of proteolytic, lioplytic, psychrotrophic and β-haemolytic activities. The recovered *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* exhibited 100% resistance towards Ampicillin, Amoxicillin and Erythromycin antibiotics, while, they exhibited 100% sensitivity towards Ciprofloxacin. The public health hazards of occurrence of *Aeromonas spp.* in milk and its products as well as the suggestive control measures were discussed.

Key words: *Aeromonas hydrophila,* Raw Milk, Public Health Hazard

INTRODUCTION

Milk is an excellent medium for the growth of numerous microbes which produce consequential spoilage of the milk and various milk products or food-borne pathogens to the consumers (Oliver *et al.*, 2005). Aeromonads are autochthonous to the aquatic environment (Aboulhamd, 2010) and are also common contaminants in diverse variety of foods like fish, sea foods, raw and cooked meats, poultry, milk and milk products, eggs and vegetables (Sharma and Kumar 2011). Scoaris *et al.* (2008) showed that, Aeromonads are efficient colonizers of surfaces and are an important constituent of bacterial biofilms in both water distribution systems and food processing environments.

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Isolation of *Aeromonas hydrophila* from water and food sources, and the increasing resistance of this organism to antibiotics and chlorination in water, presents a significant threat to public health (Chang *et al.*, 2008). Also, *Aeromonas species* have also been linked to both food and water-borne diseases in different parts of the world especially developing countries due to poor personal hygiene and lack of quality water (Odeyemi and Ahmad, 2014). Moreover, *Aeromonas hydrophila* and *Aeromonas caviae* are considered major pathogens most commonly implicated in human intestinal infections (Van Gravenitz, 2007) and also account for more than 95% of all blood-borne infections (Ghenghesh *et al.*, 2008). Furthermore, Five types of diarrhea of *Aeromonas* related gastroenteritis, secretory (acute watery diarrhea often with vomiting), dysenteric (accompanied by blood and mucus in the stool), chronic (lasting longer than 10 days), choleric (rice water stools) and travelers were reported (Janda and Duffey, 1988).

Extra-intestinal infections due to *Aeromonas species* as fatal bacteremic pneumonia, orbital cellulitis, fatal *Aeromonas hydrophila* infection of soft tissue in a

cirrhotic patient, epidural abscess, wound infection, prostatitis, septic shock, ecthyma gangrenosum, diabetic foot, fatal *Aeromonas hydrophila* myonecrosis and sepsis were reported (Kao *et al.*, 2003; Chou *et al.,* 2004; Liu *et al.,* 2005; Tsai *et al.*, 2005; Easow and Tuladhar, 2007; Avolio *et al.*, 2009; Talan *et al.*, 2014 and Yumoto *et al.*, 2014).

It has been observed that some strains of *Aeromonas* are enteropathogenic and possess virulence factors as enterotoxins, cytotoxins, haemolysins and invasins and this association between haemolysin production and enterotoxicity is none other than the *Aeromonas* sp. which can grow and release enterotoxin and haemolysin even at fridge temperature conditions (Sharma and Kumar, 2012). Antimicrobial resistance among enteric pathogens is a serious problem in developing countries where there is a high frequency of gastroenteric illness and many antibiotics fall routinely into inadequate use. Antibiotic resistance is particularly relevant in pathogenic *Aeromonas species* in which, besides the classical resistance to β-lactamic antibiotics, multiple-resistance has been frequently identified (Vila *et al.*, 2002). These bacteria can receive and transfer antibiotic resistance genes to other Gram negative bacteria (Marchandin *et al.*, 2003).

Due to the public health hazard of *Aeromonas species*, this study aimed to detect these microorganisms in raw milk and some milk products consumed in Assiut city, Egypt. Also, to determine the antimicrobial resistance, proteolytic, lioplytic, psychrotrophic and haemolytic activities of the isolated organisms. Moreover, confirmation of *Aeromonas hydrophila* strains by detection of *16S* rRNA gene and determination of haemolysin (*ahh*1) and aerolysin (*aer*A) virulence genes of isolated strains by PCR assay were assessed.

MATERIALS AND METHODS

A total of 100 raw milk, kareish cheese (traditional Egyptian cheese made from raw skimmed milk), ice cream and baladi yoghurt (25 samples, each) were collected from dairies and street peddlers in Assiut city, Egypt. The samples were collected in clean and sterile plastic bags in an ice-box and transferred rapidly as soon as possible to the laboratory for bacteriological examination.

A) **Preparation of samples:**

The apparently normal raw milk samples were mixed thoroughly and tested for heat treatment by Storch test according to Lampert (1975) before being subjected to examination. Ten ml from liquid samples and 10g from solid samples were added individually to 90 ml of 0.1% sterile peptone water. Ten-fold serial dilutions from each sample were done up to 10^6 (A.P.H.A., 2001).

B) Bacteriological examination of the prepared samples:

1) Enumeration of *Aeromonas hydrophila*, using m-*Aeromonas* selective agar, according to Palumbo *et al.* (2001): From each dilutions, 0.1 ml was spread over m-*Aeromonas* selective agar containing 10 mg ampicillin/liter with sterile bent glass rod and incubated at 28 ^{o}C for 24 hours. Then the numbers of isolated *Aeromonas* were calculated and typical colonies were picked into nutrient agar slants for biochemical identification.

2) Application of PCR for identification of *16S* rRNA, aerolysin (*aer*A) and haemolysin (*ahh*1) virulence genes of *Aeromonas hydrophila:-*

a) Primer sequences of *Aeromonas hydrophila* used for PCR identification system: Application of PCR for identification of *16S* rRNA, aerolysin (*aer*A) and haemolysin (*ahh*1) virulence genes of *Aeromonas hydrophila* was performed essentially by using primers (Pharmacia Biotech) as shown in the following table:

Target genes	Primers	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
16S rRNA	AHH1 (F)	5′ GGGAGTGCCTTCGGGAATCAGA ′3	356	**Stratev *et al.* (2016)**
	AHH1 (R)	5′ TCACCGCAACATTCTGATTTG ′3		
*aer*A	AH-aerA (F)	5′ CAAGAACAAGTTCAAGTGGCCA ′3	309	
	AH-aerA (R)	5′ ACGAAGGTGTGGTTCCAGT ′3		
*ahh*1	A16S (F)	5′ GCCGAGCGCCCAGAAGGTGAGTT′3	130	
	A16S (R)	5′ GAGCGGCTGGATGCGGTTGT ′3		

b) DNA Extraction using QIA amp kit (Shah *et al.*, 2009): After sub-culturing of *Aeromonas hydrophila* on starch ampicilin agar, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100 ºC for 20 minutes. Accurately, DNA was extracted from all isolates by using QIAamp kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions). All DNA extracts were stored at -20 ºC until used.

c) Amplification reaction of *Aeromonas hydrophila* (Wang *et al.*, 2003): The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). Multiplex PCR assays were adopted by using 25 µl of the reaction mixture contained 2X AmpliTaq DNA polymerase (Perkin-Elmer) – 12.5 µl, and 1.0 µl of each *ahh1* and *aer*A primers, 0.2 µl of *16S* rRNA primers and 1 µl extracted DNA. Amplification conditions consisted of an initial denaturation at 95 °C for 5 min., 50 cycles at 95 °C for 30 sec., 59 °C for 30 sec., 72° C for 30 sec., followed by final elongation at 72° C for 7 min. Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1X TAE buffer (0.04 M Tris, 0.02 M Acetic acid, 0.002 M Na2 EDTA) at 100 V for 45 min with 8 µl PCR product. Finally, the gel was stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

3) Detection of proteolytic activity of *Aeromonas spp*. according Swift *et al.* (1999): This was performed by placing aseptically loopful of culture on the center of the 10 % of skim milk agar plate and spreading it in a circular fashion to cover an area about 5 to 18mm in diameter; then the plates were incubated in an inverted position at 37 ºC for 24 to 48 h. Clearing the cloudy agar (zone of proteolysis) indicated a positive result.

4) Detection of lipolytic activity of *Aeromonas spp*. according to Harrigan (1998): The strains were subcultured in tributyrin agar (Plate Count Agar supplemented with 1% tributyrin) and then incubated at 37 °C for 48 h. The colonies were considered positive when a precipitation halo formed around the colony, indicating the release of enzymes into the growth medium.

5) Determination of psychrotrophic activity of *Aeromonas spp*., using Standard Plate count Agar, according to A.P.H.A. (2001): The isolated *Aeromonas spp.* strains were inoculated on Standard Plate Count Agar and incubated at 7 ^{o}C for 10 days then examined for *Aeromonas* growth.

6) Determination of haemolytic activity of *Aeromonas spp.* according to Swift *et al.* (1999): Haemolytic positive isolates were identified by the presence of clear (β-haemolysis) halos around the colonies grown at 37 °C for 24 h on TSA agar (Difco) containing 5 % rabbit blood.

7) Antibiogram of *Aeromonas species* according to CLSI (2011): *Aeromonas spp*. strains isolated in the present study were subjected to susceptibility testing against 8 antimicrobials commonly used. Susceptibility was determined by the disk-diffusion technique of Kirby-Bauer on Mueller-Hinton agar plates with inocula adjusted to an optical density of 0.5 McFarland standard units. Disks containing Ampicillin (AMP 10µg), Amoxicillin (AML 10µg), Kanamycin (K 30µg), Ceftriaxone (CRO 30µg), tetracycline (TE 30µg), Erythromycin (E 15µg), Trimethoprim/Sulfamethoxazole (SXT 25µg) and ciprofloxacin (CIP 5µg) were used. After 24 h incubation at 30°C, organisms were classified as Sensitive (S), Intermediately resistant (I) or Resistant (R) on the basis of the size of the zone of bacteria growth inhibition.

RESULTS

Table 1: *Aeromonas spp*. count in raw milk, kareish cheese, ice-cream and yoghurt (n= 25).

Type of sample	*Aeromonas spp*. count (cfu/ml or cfu/g)					
	Positive countable samples		Min.	Max.	Average	± SE
	No.	%				
Raw milk	9	36	< 100	1.2×10^{6}	1.0×10^{5}	5.6×10^{4}
Kareish cheese	8	32	< 100	6.0×10^{5}	3.2×10^{4}	2.4×10^{4}
Ice cream	6	24	< 100	7.0×10^{3}	5.0×10^{2}	2.9×10^{2}
Baladi yoghurt	-	0.0	< 100	< 100	< 100	-

< 100 means negative samples

Table 2: Incidence of countable *Aeromonas spp.* in raw milk, kariesh cheese and ice-cream samples (n= 25).

Type of sample	Positive results					
	Aeromonas hydrophila		*Aeromonas caviae*		*Aeromonas sobria*	
	No.	%	No.	%	No.	%
Raw milk	4	16	3	12	2	8
Kareish cheese	3	12	4	16	1	4
Ice-cream	2	8	3	12	1	4

Table 3: Proteolytic and lipolytic activities of *Aeromonas spp.* detected in raw milk, kareish cheese and ice-cream samples.

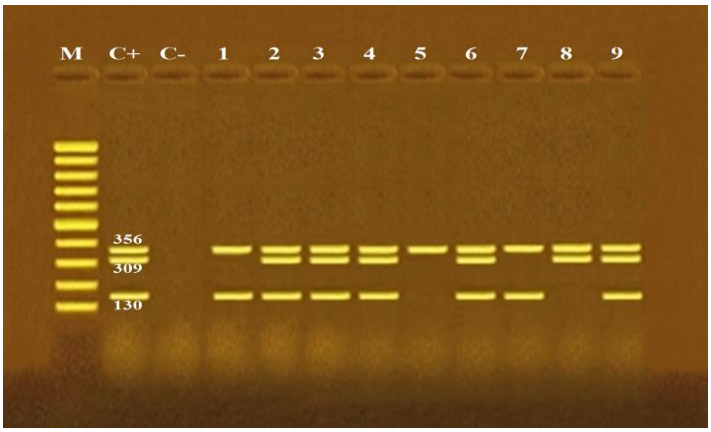
Tested organisms	No. of tested isolates	Proteolytic activity		Lipolytic activity	
		Positive samples		Positive samples	
		No.	%	No.	%
Aeromonas hydrophila	9	9	100	9	100
Aeromonas caviae	10	10	100	10	100
Aeromonas sobria	4	4	100	4	100

Table 4: Psychrotrophic and haemolytic activities of *Aeromonas spp.* detected in raw milk, kareish cheese and ice-cream samples.

Tested organisms	No. of tested isolates	Psychrotrophic activity		β-haemolysis	
		Positive samples		Positive samples	
		No.	%	No.	%
Aeromonas hydrophila	9	9	100	9	100
Aeromonas caviae	10	10	100	10	100
Aeromonas sobria	4	4	100	4	100

Table 5: Incidence of *16S* rRNA, aerolysin (*aer*A) and haemolysin (*ahh*1) virulence genes in isolated *Aeromonas hydrophila* organism (n= 9).

Tested organisms	*16S* rRNA gene		Aerolysin (*aer*A) gene		Haemolysin (*ahh*1) gene	
	Positive		Positive		Positive	
	No.	%	No.	%	No.	%
Aeromonas hydrophila	9	100	6	66.67	7	77.78



Photograph (1): Agarose gel electrophoresis of multiplex PCR of *16S* rRNA (356 bp), *aer*A (309 bp) and *ahh*l (130 bp) genes for characterization of *Aeromonas hydrophila.*

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *Aeromonas hydrophila* for *16S* rRNA, *aer*A and *ahh*l genes.
Lane C-: Control negative.
Lanes 2, 3, 4, 6 & 9: Positive *Aeromonas hydrophila* for *16S* rRNA, *aer*A and *ahh*l genes.
Lanes 1 & 7: Positive *Aeromonas hydrophila* strains for *16S* rRNA and *ahh*l genes.
Lane 8: Positive *Aeromonas hydrophila* strain for *16S* rRNA and *aer*A genes.
Lane 5: Positive *Aeromonas hydrophila* strain for *16S* rRNA gene.

Table 6: Antibiogram of *Aeromonas spp*. isolated from raw milk and some milk products.

Antibiotics	*Aeromonas hydrophila* (n=9)						*Aeromonas caviae* (n= 10)						*Aeromonas sobria* (n= 4)					
	Sensitive		Intermediate		Resist		Sensitive		Intermediate		Resist		Sensitive		Intermediate		Resist	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	-	0.0	-	0.0	9	100	-	0.0	-	0.0	10	100	-	0.0	-	0.0	4	100
Amoxicillin	-	0.0	-	0.0	9	100	-	0.0	-	0.0	10	100	-	0.0	-	0.0	4	100
Kanamycin	2	22.22	3	33.33	4	44.44	4	40	5	50	1	10	3	75	1	25	-	0.0
Ceftriaxone	5	55.56	1	11.11	3	33.33	4	40	3	30	3	30	3	75	1	25	-	0.0
Tetracycline	4	44.44	1	11.11	4	44.44	5	50	-	0.0	5	50	1	25	1	25	2	50
Erythromycin	-	0.0	-	0.0	9	100	-	0.0	-	0.0	10	100	-	0.0	-	0.0	4	100
TSX*	-	0.0	2	22.22	7	77.78	2	20	3	30	5	50	2	50	2	50	-	0.0
Ciprofloxacin	9	100	-	0.0	-	0.0	10	100	-	0.0	-	0.0	4	100	-	0.0	-	100

*Trimethoprim-Sulfamethoxazole

DISCUSSION

The illustrated results in Table 1 revealed that, the incidence of counted *Aeromonas spp*. in the examined raw milk samples was 36%, with counts ranging from < 100 to 1.2×10^6 and with an average count of 1.0×10^5 cfu/ml. Lower incidences (8.7, 26 and 32%) were estimated by Amer *et al.* (2008); Enany *et al.* (2013) and Ahmed *et al.* (2014), respectively. On the other hand, higher incidences (40, 49.2, 86.7, 46.7 and 58%) were detected by El-Shorbagy and Al-Ganzoury (2002); Yucel *et al.* (2005); Korashy (2006); El-Taib and Mohamed (2010) and Eid *et al.* (2013), respectively. It is worth mentioning that, *Aeromonas* organisms can invade udder tissues; multiply in mammary tissues and subsequently discharge in milk. Also, the contaminated water used for washing milking equipments is considered as a significant source of contamination. Therefore, presence of *Aeromonas* in a high level in raw milk samples in this study is indicative to bad hygienic measures of milk production and distribution (Korashy, 2006).

The incidence of counted *Aeromonas spp*. in the examined kareish cheese samples was 32%, with a count ranging from < 100 to 6.0×10^5 and with an average count of 3.2×10^4cfu/g (Table 1). Lower incidence of 12% was reported by Enany *et al.* (2013), whereas, higher incidences (92, 58, 70 and

70%) were revealed by Al-Ashmawy (2000); Effat *et al.* (2000); Korashy (2006) and Eid *et al.* (2013), respectively. The presence of *Aeromonas species* in kareish cheese could be attributed to the bad quality of the raw milk used, the unsanitary manufacturing practices, improper handling and distribution. Also, the kareish cheese manufacturing process itself does not appear to be deleterious to *Aeromonas spp*.

Moreover, the incidence of counted *Aeromonas spp*. in ice cream samples was 24%, with a count ranging from < 100 to 7.0×10^3 and with an average count of 5.0×10^2cfu/g (Table 1). Lower incidence of 18% was estimated by Sharef *et al.* (2006). The presence of *Aeromonas spp*. in ice cream samples indicated the unhygienic measures during preparation and distribution of the products. *Aeromonas spp*. isolated from human stool samples in percentage of 3.4% (Aslani and Alikhani, 2004), therefore, presence of *Aeromonas hydrophila* in ice cream may indicate faecal contamination from workers. Furthermore, good hygienic practices and good personal hygiene must be applied to produce products save for human consumption.

Aeromonassp. was not detected in baladi yoghurt samples in this study (Table 1). Motlagh *et al.* (1991) reported that, starter culture bacteria in yoghurt can produce antimicrobial activity against *Aeromonas hydrophila* and they declared that, diacetyl had some bactericidal activity against the tested strains. This observation could explain why *Aeromonas* was not detected in yoghurt samples in this study. Moreover, low pH of yoghurt may have an inhibitory effect on *Aeromonas* organisms or other factors which need further investigation.

The incidence of *Aeromonas hydrophila* in the examined raw milk samples was 16% (Table 2). Similar incidences (15.9 and 17.1%) were found by Melas *et al.* (1999) and Subashkumar *et al.* (2006), respectively. Whereas, lower incidences (9 and 7%) were detected by El-Leboudy *et al.* (2014) and Alrazakkazal and Abdullah (2016), respectively. On the other hand, higher incidences (36 and 24%) were revealed by Abdel-Raouf and Naima (2011) and Zeinhom and Abdel-Latef (2014), respectively. *Aeromonas hydrophila* isolated from feaces of normal cow in percentage of 21.1% (Agarwal *et al.*, 2000), therefore, presence of *Aeromonas hydrophila* in raw milk may indicate faecal contamination of milk.

Table 2 revealed that, the incidence of *Aeromonas hydrophila* in the examined kareish cheese samples was 12%. Higher incidence of 32.5% was obtained by Nazem *et al.* (2010). While for the examined ice cream samples, the incidence of *Aeromonas hydrophila* was 8%. Higher incidence of 40% was estimated by Nazem *et al.* (2010). From the aforementioned results, raw milk, kareish cheese and ice cream may cause risks for public health from *Aeromonas hydrophila* infection, especially for immune-compromised person, children and aged. Hence, there is need for public enlightenment, campaign and general education to assist in curtailing the outbreak of diseases in human through ingestion of the bacteria along with milk and milk products. Preventive method should be taken during food preparation; good personal hygiene and proper sanitation procedure should always be use to prevent human exposure to this disease.

The entire isolated *Aeromonas hydrophila* organism detected in raw milk, kareish cheese and ice cream (9 strains), in this study, were confirmed by PCR assay for detection of *16S* rRNA gene and all the tested isolate were harboring this gene (Table 5 and Photograph 1). The *16S* rRNA gene was included as an internal control and has become the gold standard method for definitive species identification (Wang *et al.*, 2003 and Geetha and Michael, 2015).

Aeromonas caviae was isolated from raw milk samples in percentage of 12% (Table 2). Similar incidence of 13% was revealed by Melas *et al.* (1999). For kareish cheese samples, the incidence of *Aeromonas caviae* was 16% (Table 2) and this result was lower than that detected by Effat *et al.* (2000). Concerning ice cream samples, the incidence of *Aeromonas caviae* was 12% (Table 2).

The incidence of *Aeromonas sobria* in the examined raw milk samples was 8% (Table 2). Lower result (3.6%) was obtained by Melas *et al.* (1999), whereas higher incidence was estimated by Akan *et al.* (1996). In contrary, Seker *et al.* (2015) could not isolate the organism from milk samples. Regarding to kareish cheese samples, the incidence of *Aeromonas sobria* was 4% (Table 2). Higher incidences were detected by Effat *et al.* (2000) and Korashy (2006). With regard to ice cream samples, the incidence of *Aeromonas sobria* was 4% (Table 2). The aforementioned results revealed that, *Aeromonas hydrophila* and *Aeromonas caviae* organisms were the most predominant strains isolated from raw milk, kareish cheese and ice cream samples followed by *Aeromonas sobria*.

The isolated *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* organisms in this study were tested for its virulence properties by proteolytic and lipolytic assay and all the tested isolates exhibited 100% proteolytic and lipolytic activities (Table 3). Similar results were observed by Castro-Escarpulli *et al.* (2003), Singh *et al.* (2010), Al-Fathawy and Al-Ammar (2013), Al-Oqaili *et al.* (2016) and Simon *et al.* (2016).

It is worth mentioning that, Proteases play an important role in gastroenteritis by proteolytic

activation of the haemolysin and also, it plays an important role in pathogenesis by providing nutrients for the bacteria during the colonization and the invasion of the bacteria in the host (Santos *et al.,* 1999 and Zhu *et al.,* 2007). Furthermore, Lipases have been considered to be important for bacterial nutrition and also play role as virulence factors by interacting with human leukocytes or by affecting several immune system functions through free fatty acids generated by lipolytic activity. Extracellular lipases secreted by *Aeromonas spp*. actively participate in the alteration of the host plasma membrane and thus increase the severity of infection (Pemberton *et al.,* 1997).

Nevertheless, proteolytic and lioplytic properties of the Aeromonads in this study may cause bitterness, rancidity and other defects in milk and milk products. Therefore, good hygiene masseurs and strict eradication programs must be applied in dairy plant to produce milk and milk products save for human consumption and of good keeping quality.

Results in Table 4 revealed that, all the tested *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* organisms had 100% psychrotrophic properties. Lower results were estimated by Ahmed *et al.* (2014). The psychrotrophic properties of Aeromonads are aiding factors for survival and multiplication of the organisms in foods even at refrigerator temperature consequence health risks to consumers.

Table 4 showed that, all the tested *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* had 100% β-haemolytic activities. Similar results were estimated by Subashkumar *et al.* (2006); Al-Fathawy and Al-Ammar (2013); Joseph *et al.* (2013); Taj-Aldeen *et al.* (2014); Al-Oqaili *et al.* (2016) and Simon *et al.* (2016). On the other hand, lower results were revealed by Majeed (2011); Manna *et al.* (2013); Amsaveni *et al.* (2014); Mansour *et al.* (2014) and Sharma *et al.* (2015).

Haemolytic activity is an index of pathogenicity and the relationship between the production of haemolysin and the enterotoxigenicity in *Aeromonas spp*. well documented (Obi *et al.,* 2007). Nevertheless, all the isolated Aeromonads in this study were haemolytic on blood agar indicating their pathogenic nature and had potential public health significance in food of animal origin. Therefore it is concluded that, haemolytic activity on blood agar is simpler and an easier way than PCR assay for detection Aeromonads pathogenicity and also economical to use especially for developing countries.

The illustrated results in Table 5 and Photograph 1 revealed that, the incidences of *aer*A and *ahh*1 genes in the tested *Aeromonas hydrophila* were 66.67 and 77.78%, respectively. Similar incidence was detected by Al-Fathawy and Al-Ammar (2013), whereas lower result was estimated by Yousr *et al.* (2007). In contrary, higher incidences were recorded by Rather *et al.* (2014) and Simon *et al.* (2016). The *aer*A and *ahh*1 genes are cytotoxic and these two toxins contribute to development of severe diarrhoea in humans because of their synergistic effects (Guerra *et al.,* 2007).

From Photograph 1 it is clear that, the strain of *Aeromonas hydrophila* in Lane 5 had neither *aer*A gene nor *ahh*1 gene while it exhibited haemolytic activity on blood agar (Table 4). Therefore one can easily conclude that, haemolytic activity of *Aeromonas hydrophila* may be expressed by genes other than *aer*A and *ahh*1 genes and this observation need further investigations.

The tabulated results in Table 6 showed that, all the tested *Aeromonas hydrophila*, Aeromonas *caviae* and *Aeromonas sobria* organisms exhibited 100% resistance towards Ampicillin, Amoxicillin and Erythromycin antibiotics. Similar results were revealed by Kannan *et al.* (2001); Majeed *et al.* (2011); Enany *et al.* (2013); Furmank-Blaszk (2014) and Sharma *et al.* (2015). The tested strains of *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* exhibited sensitivity to Kanamycin in percentages of 22.22, 40 and 75%, respectively. Similar results were obtained by Subashkumar *et al.* (2006) and Didugu *et al.* (2016).

The isolated *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* organisms were sensitive towards Ceftriaxon in percentages of 55.56, 40 and 75%, respectively (Table 6). Similar results were observed by Ragunathan *et al.* (2012) and Eid*et al.* (2013). For Tetracycline, the *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* organisms exhibited 44.44, 50 and 25% sensitivity, respectively (Table 6) and these results simulated what obtained by Kaskhedikar and Chhabra (2010). While for Trimethoprim-Sulfamethoxazole, the tested organisms of *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* showed sensitivity percentages of 0.0, 20 and 50%, respectively and these were lower than what detected by Aboulhamd (2010). Concerning Ciprofloxacin, all the tested *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* strains were sensitive in percentage of 100% and theses results go parallel with what estimated by Castro-Escarpulli *et al.* (2003); Yucel and Citak (2003); Guerra *et al.* (2007) and Kaskhedikar and Chhabra (2010).

The problem of Aeromonads resistance towards multiple antibiotics causes an increase in the risk of treatment failure and cost for antimicrobial therapy and hospitalization, while the range of therapeutic options decreases (Wen-Chien *et al.,* 1998).

Moreover, the emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immune-suppressed patients.

CONCLUSION

This study revealed that, raw milk, kareish cheese and ice cream that retailed in Assiut city, are largely contaminated with Aeromonads microorganisms. The isolated *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* exhibited virulence properties on the bases of proteolytic, lioplytic, psychrotrophic and haemolytic activities and in addition to the presence of *aer*A and *ahh*1 genes in *Aeromonas hydrophila.* Resistance of the recovered Aeromonads towards different varieties of antibiotics was observed and Ciprofloxacin was the most efficient antibiotic against *Aeromonas species*. Good hygienic control measures must be applied in dairy farms in addition to the use of raw milk of good bacteriological quality before manufacturing of different varieties of milk products.

Dedication: To the soul of Dr. N.H. Makar, Senior Researcher, Bacteriology Department, Animal Health Research Institute, Assiut Lab., who died before accomplishing this work.

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الكشف عن الايروموناس هيدروفيلا في اللبن الخام وبعض منتجات الألبان مع الإشارة إلي خطورتها علي الصحة العامة

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هدفت هذه الدراسة للكشف عن وجود ميكروبات الايروموناس في اللبن الخام وبعض منتجاته فقد تم تجميع عدد ١٠٠ عينة من اللبن الخام والجبن القريش والايس كريم والزبادي البلدي (٢٥ عينة لكل نوع) من محلات الألبان والباعة الجائلين بمدية أسيوط لفحصها بكتريولوجياً. فقد كانت نسب وجود ميكروبات الايروموناس في اللبن الخام والجبن القريش والايس كريم والزبادي البلدي هي ٣٦ ، ٣٢ ، ٢٤ ، ٠٪ علي الترتيب وبمتوسطات عد علي التوالي هي ١×١٠^{٥} ، ٣.٢×١٠^{٤} ، ٥×١٠^{٢} و< ١٠٠ مستعمرة/مل. وكانت نسب وجود بكتريا الايروموناس هيدروفيلا في اللبن الخام والجبن القريش والايس كريم هي ١٦ ، ١٢ و ٨٪ علي الترتيب ، بينما كانت نسب وجود بكتريا الايروموناس كيفي في هذه العينات هي علي التوالي ١٢ ، ١٦ و ١٢٪ في حين انة كانت نسب وجود بكتريا الايروموناس سوبريا في تلك العينات هي ٨ ، ٤ و ٤٪ علي الترتيب. وقد كانت جميع عينات الزبادي البلدي في هذه الدراسة خالية من ميكروبات الايروموناس. وقد تم تأكيد نوع الايروموناس هيدروفيلا المعزولة باستخدام اختبار إنزيم البلمرة المتسلسل للكشف عن وجود جين *16S rRNA* وقد كانت جميع العزلات المختبرة تحوي هذا الجين بنسبة ١٠٠٪. في حين انة كانت نسب وجود جينات الضراوة*aer*A و *ahh*1 هي ٦٦.٦٧ و ٧٧.٧٨٪ علي الترتيب. وتم اختبار ضراوة ميكروبات الايروموناس هيدروفيلا والايروموناس كيفي والايروموناس سوبريا باستخدام اختبارت تحلل البروتين ، تحلل الدهون ، النمو في درجة الحراة المنخفضة والقدرة علي تحليل كرات الدم الحمراء فقد أعطت جميع هذه الميكروبات ضراوة بنسبة ١٠٠٪. وقد تم اختبار مقاومة ميكروبات الايروموناس هيدروفيلا والايروموناس كيفي والايروموناس سوبريا للمضادات الحيوية وأعطت جميع هذه الميكروبات مقاومة بنسبة ١٠٠٪ لكل من المضاد الحيوي الامبسيلين والاموكسيسيلن والارثروماسين ، في حين كانت جميع هذه الميكروبات حساسة بنسبة ١٠٠٪ للمضاد الحيوي السبروفلوكساسين. تمت مناقشة مصادر تلوث الالبان بهذه الميكروبات ، الاهمية الصحية للمعزولات وكذلك الضوابط والاحتياطات الواجب إتباعها للحد من تلوث الالبان ومنتجاتها بها.